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Differential role of the 5-HT_{1A} receptor in aggressive and non-aggressive mice: An across-strain comparison

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Abstract

Differential role of the 5-HT_{1A} receptor in aggressive and non-aggressive mice: an across-strain comparison. PHYSIOL BEHAV 00(0) 000-000, 2006. According to the serotonin (5-HT)-deficiency hypothesis of aggression, highly aggressive individuals are characterized by low brain 5-HT neurotransmission. Key regulatory mechanisms acting on the serotonergic neuron involve the activation of the somatodendritic inhibitory 5-HT_{1A} autoreceptor (short feedback loop) and/or the activation of postsynaptic 5-HT_{1A} receptors expressed on neurons in cortico-limbic areas (long feedback loop). In this study, we examined whether low serotonin neurotransmission is associated with enhanced 5-HT_{1A} (auto)receptor activity in highly aggressive animals. Male mice (SAL-LAL, TA-TNA, NC900-NC100) obtained through different artificial-selection breeding programs for aggression were observed in a resident–intruder test. The prefrontal cortex level of 5-HT and its metabolite 5-HIAA were determined by means of HPLC. The activity of the 5-HT_{1A} receptors was assessed by means of the hypothermic response to the selective 5-HT_{1A} agonists S-15535 (preferential autoreceptor agonist) and 8-OHDPAT (full pre- and postsynaptic receptor agonist). Highly aggressive mice had lower serotonin levels in the prefrontal cortex and two out of three aggressive strains had higher 5-HT_{1A} (auto)receptor sensitivity. The results strengthen the validity of the serotonin-deficiency hypothesis of aggression and suggest that chronic exaggerated activity of the 5-HT_{1A} receptor may be a causative link in the neural cascade of events leading to 5-HT hypofunction in aggressive individuals.

Keywords: Aggression; Resident–intruder; Serotonin, 5-HT_{1A}; Mice; Selection lines

1. Introduction

The neurobiology of aggressive behavior includes a central role for brain serotonin (5-HT) neurotransmission. The most favored idea is the "5-HT deficiency" hypothesis of aggression [1–4], stating that high trait-like levels of aggressive behavior are associated with low 5-HT neurotransmission activity. This hypothesis implies that individuals of high and low aggressiveness might somehow differ in the regulation of 5-HT neurotransmission.

An important regulatory mechanism of the serotonergic system is represented by 5-HT_{1A} receptors [5,6]. These are located pre-synaptically as autoreceptors on the soma and the dendrites of serotonergic neurons, as well as postsynaptically on non-serotonergic neurons in several corticolimbic areas that receive 5-HT terminals [7,8]. Activation of somatodendritic 5-HT_{1A} autoreceptors by 5-HT or 5-HT_{1A} agonists potently decreases the firing rate of 5-HT neurons and consequently leads to the suppression of 5-HT synthesis, 5-HT turnover and 5-HT release in the diverse projection areas[6,9,10]. In addition to this local somatodendritic autoreceptor short negative-feedback loop, a long feedback loop also exists, which entails postsynaptic 5-HT_{1A} receptor activation to inhibit 5-HT neuron-firing activity via reducing excitatory afferent input [6,11,12]. Furthermore, it has been shown that 5-HT_{1A} agonists exert potent anti-aggressive properties in rodents [13–15], and in particular in animals that show high and/or escalated levels of aggressive behavior [16–19]. Therefore, it can be hypothesized that an impairment of serotonergic neurotransmission in high aggressive animals may be caused by excessive inhibitory 5-HT_{1A} receptor activity.

Several years ago, three independent artificial selection programs in the Netherlands, Finland and North Carolina were

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carried out in mice. This has lead to the generation of three highly aggressive lines (SAL=Short Attack Latency, TA=Turku Aggressive, NC900=North Carolina 900) and three "docile" lines (LAL=Long Attack Latency, TNA=Turku Non Aggressive, NC100=North Carolina 100) ([20–22]; for review see [23]). Several studies suggest that these pairs of highly and weakly aggressive lines show similar differences in the serotonergic system. The overall brain content of serotonin is lower in SAL than in LAL mice [24]. In another study [25] the amounts of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and the 5-HIAA/5-HT ratio were examined in different brain regions and in different phases of the daily light/dark cycle. The 5-HT baseline level in the brain stem of SAL mice was lower than that for LAL during the light phase, while the metabolic ratio was lower in the striatum and in the amygdala. In a second experiment, the 5-HT content of saline-treated SAL mice was lower in the prefrontal cortex, striatum, hippocampus and amygdala than of saline-treated LAL mice [25]. Neurochemical studies in the Finnish selected mice showed similar results. Mice from the aggressive line (TA) had 19% lower levels of serotonin in the prefrontal cortex than mice from the non-aggressive line (TNA) [26]. An initial study on the regulation of the serotonergic system found higher 5-HT_{1A} receptor ligand binding in SAL than LAL in the dentate gyrus, CA1, lateral septum and frontal cortex, and higher 5-HT_{1A} mRNA in the CA1 and dentate gyrus of the hippocampus [27]. Further analysis [25] replicated some of these findings. However, no difference was found in 5-HT_{1A} mRNA expression and binding capacity in the prefrontal cortex and lateral septum, probably due to a circadian fluctuation affecting the results. Moreover, the behavioral response tested in the forced-swimming test to 8-OH-DPAT, a selective full pre- and postsynaptic 5-HT_{1A} agonist, and to S-15535, a preferential pre-synaptic 5-HT_{1A} agonist, was different between the two lines, suggesting a different sensitivity of this receptor. Indeed, the hypothermic response to the full 5-HT_{1A} receptor agonist alnespirone, as measured with a rectal probe, was higher in SAL than in LAL [28], indicating a more sensitive 5-HT_{1A} receptor functioning in the aggressive line. In line with these findings, electrophysiology experiments on hippocampal slices showed that the serotonin-induced membrane hyperpolarization was more pronounced in SAL mice than in LAL mice [29]. To our knowledge, no investigation of the serotonergic system in the NC900 and NC100 lines has been performed.

This study focuses on the involvement of the 5-HT_{1A} autoreceptor regulation of serotonergic activity in aggressive behavior. The three genetic selection lines of high and low aggressive behavior were used to study the general validity of this putative autoreceptor-mediated control in aggression.

According to the "serotonin-deficiency" hypothesis of impulsive and violent aggression, we would expect high aggression to correlate with low serotonin and/or low serotonin metabolism. To relate aggressiveness levels to serotonin, the behavior of the mice was observed in a resident–intruder paradigm and the tissue concentrations of 5-HT, 5-HIAA and the 5-HIAA/5-HT ratio in the prefrontal cortex were determined. In order to assess the functionality of the pre- and postsynaptic 5-HT_{1A} receptors, highly aggressive and non-aggressive mice were injected with the selective 5-HT_{1A} agonists S-15535 [30] and 8-OH-DPAT [31], and a decrease in body temperature due to the drugs was the readout for the sensitivity of the receptor. In rats, the novel ligand S-15535 has been described as a suppressor of the stress-induced hyperthermia (SIH) due to the injection [19], while 8-OH-DPAT is well known to cause hypothermia [19,32,33]. A tonically higher functional sensitivity of the 5-HT_{1A} receptor would explain a stronger inhibition of the serotonergic output from the raphe nuclei.

2. Materials and methods

2.1. Animals

Male mice (*Mus musculus domesticus*) from six different strains obtained by means of three selective breeding programs for offensive aggression were used. Short Attack Latency (SAL) and Long Attack Latency (LAL) were inbred strains derived from a wild population in Groningen, the Netherlands [20]. Turku aggressive (TA) and non-aggressive (TNA) were outbred strains obtained through artificial selection on Swiss albino mice in Turku, Finland [21]. NC900 (aggressive) and NC100 (non-aggressive) were outbred strains derived from selection on ICR mice in North Carolina [22]. The animals were bred in our laboratory and kept in unisexual familiar groups until weaning in perspex cages (17×11×13 cm). The mice were weaned at 3–4 weeks of age and housed with a female of the same line at the age of 6–8 weeks, in order to avoid social isolation and inter-male competition. During all experiments each male–female pair was housed in a Makrolon Type II cage (375 cm^2) with sawdust as bedding material, shredded paper (envirodry) as nesting material and a cardboard tube as cage enrichment. Food in the form of rodent pellets (AMII, ABDiets, Woerden, The Netherlands), and water with a low chloride content were provided *ad libitum*. The animals were kept in a room with a 12:12 light/dark cycle and constant temperature (22±2 °C).

2.2. Biotelemetry

At 4–6 months of age, a group of male mice of each line was implanted with a biotelemetry transmitter for chronic core body temperature recordings. Two types were used: 3000XM-FH (Mini-Mitter, USA) and TA10ETA-F20 (DSI, St Paul, Minnesota, USA). During surgery, the animals were anesthetized with 5% isoflurane/O_2/N_2O, placed on a Harvard homoeothermic heating pad in order to prevent hypothermia due to the anesthetics, and maintained under anesthesia with 2.5% isoflurane/O_2/N_2O. A transmitter was placed inside the abdominal cavity, and in the case of TA10ETA-F20, one lead was fixed to the xyphoid process of the sternum and the other to the pectoral muscular layer in the right mediastinum. Internal suturing was performed by means of reabsorbable surgical thread, while skin was sutured with silk with a reverse-knot method, in order to prevent chewing by the animal. Natrium penicillin was injected as post-surgery antibiotic treatment.

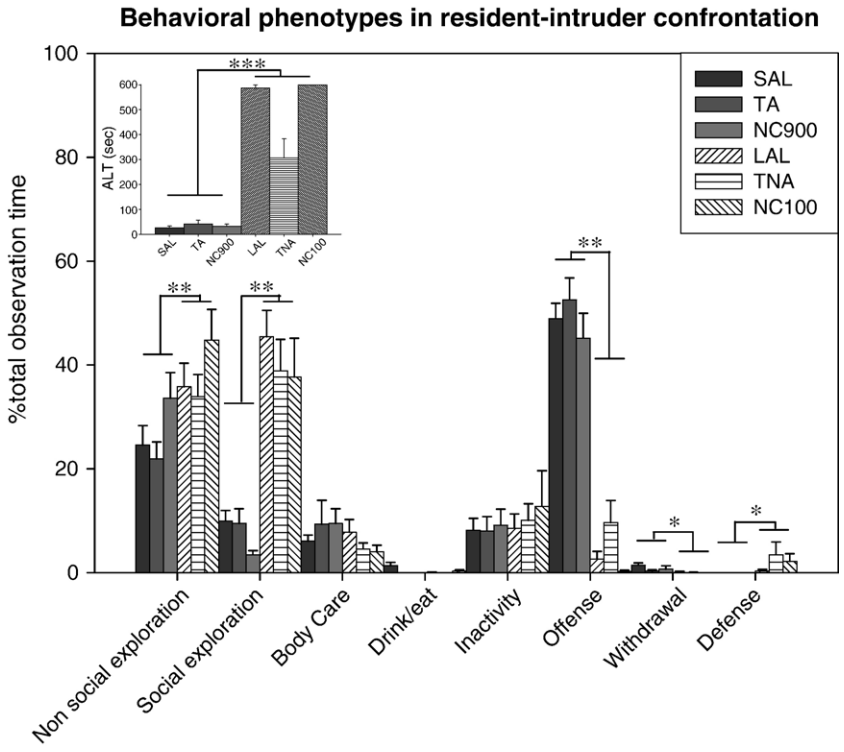

Fig. 1. Behavioral phenotype (main figure) and average attack latency time (top-inset) of SAL ($n=17$), LAL ($n=14$), TA ($n=8$), TNA ($n=9$), NC900 ($n=8$) and NC100 ($n=9$) mice during a 5-min resident–intruder confrontation (see text for explanation). Data are represented as means ± SEM. **type effect: aggressive vs. non-aggressive different at $p<0.001$, **type effect: aggressive vs. non-aggressive different at $p<0.01$, *type effect: aggressive vs. non-aggressive different at $p<0.05$.

The signal produced by each transmitter was received by an antenna/receiver board (RPC-1, Data Sciences Int., 32 × 22 × 3 cm), placed underneath the animal's cage. Each receiver was connected to a consolidation matrix (BCM-100), which was in turn connected to a PC (IBM Pentium-compatible) data-acquisition and analysis system (Dataquest Labpro™, Data Sciences). The data-acquisition parameters were set for 10-s sampling every 5 min on a 24-h basis.

The animals were allowed to recover for at least 14 days after the surgery, during which time their temperature and locomotor activity were monitored. The re-establishment and stability of circadian rhythmicity was a prerequisite for the start of the experiments.

2.3. Pharmacological challenges

Prior to the start of and 1 week after the pharmacological challenge session, each male mouse implanted for telemetry was tested in the Attack Latency Time (ALT) test according to the procedure described by Van Oortmerssen and Bakker [20], in order to confirm the aggressive or non-aggressive behavioral phenotype, which could have been affected by the surgical procedure. Behavioral results showed that the aggression trait did not change due to the pharmacological challenges.

Drug challenge tests were performed during different phases of the light/dark cycle. A wash-out period of 1–3 days between treatments was used, since 24 h are considered enough to obtain complete clearance of S-15535 and 8-OHDPAT [34,35]. Each experimental animal received a series of subcutaneous injections in randomized order, which consisted of distilled water (vehicle, 5 ml/kg of body weight), S-15535 (10 mg/kg) and 8-OH-DPAT (0.25 or 0.5 mg/kg). The volume injected was 5–10 ml/kg of body weight. The animals were distributed to different cohorts for logistic reasons. The first and the second cohorts consisted of SAL and LAL mice implanted with Mini-Mitter transmitters, whereas the third cohort included also mice from the TA, TNA, NC900 and NC100 lines, all implanted with DSI transmitters. Due to the different baseline body temperature of SAL and LAL mice between the light (first and second cohorts) and the dark phase (third cohort), these data were analyzed separately. For TA, TNA, NC900 and NC100, the data were averaged and analyzed together.

S-15535-3 methanesulfonate [(4-benzodioxan-5-yl)-1-(indan-2-yl)piperazin, lot n. EI798] was provided by Institut de Recherches Internationales Servier, France. 8-OH-DPAT [(±)-8-Hydroxy-2-(dipropylamino) tetralin hydrobromide] was obtained from Sigma-Aldrich, the Netherlands. Drugs were dissolved in distilled water (vehicle) at room temperature.

2.4. Behavioral testing: the resident–intruder paradigm

At least 1 week after the last injection, all the animals were tested for aggressive behavior using a modified version of the ALT test [20]. One day prior to the test, the males were housed in one compartment of an 80 × 30 × 30 cm partition cage with their female partners. One hour before the first test, the females were removed. The test, which was performed at the beginning of the dark phase in dim light, consisted of placing an albino male naive intruder (MAS-Gro) in one side of the cage, physically separated

from the resident male by a perforated sliding partition that allowed sensory contact. When the resident showed interest in the intruder (sniffing, approaching), or after 5 min if no interest was detected, the partition was removed to allow a physical interaction. At the first attack, the Attack Latency Time (ALT) was recorded. The intruders were not removed after the first attack, as in the original version of the test; instead, a 5-min videotape recording of the interaction for each animal was made. If there was no attack from the resident, the test was continued for a maximum of 10 min, since an ALT of 600 s would arbitrarily indicate a true non-attacking phenotype. Immediately after the test, all animals (males and females) were put back in their home cages. From the videotape recordings, and using The Observer software (Noldus Information Technology bv), the following behaviors were scored, as described by Koolhaas et al. [36] in rats and Brain [37] in mice: *digging*, *non-social investigation* (explore, rear, supported rear, scan), *social investigation* (approach, crawl over, crawl under, follow, groom, head groom, investigate, nose sniffing), *immobility*, *resting, body care* (self-grooming, wash, shake, scratch), *drinking/eating*, *attack* (charge, lunge, attack, chase), *threat* (aggressive groom, sideways offensive, upright offensive, tail rattle), *withdrawal* (retreat), and *defense*. An ethogram was created based on the following behavioral classes, which were subsequently analyzed: (1) non-social exploration (digging + non-social investigation); (2) social investigation; (3) inactivity (immobility + resting); (4) body care; (5) drinking/eating; (6) offense (attack + threat); (7) withdrawal; (8) defense.

2.5. Biochemical assay

In order to determine the 5-HT and 5-HIAA contents, all the animals were anesthetized with CO_2 and decapitated at least 2 days after the behavioral test. The brains were rapidly removed from the skull and the prefrontal cortex (PFC) removed, frozen in liquid nitrogen and stored at −80 °C. The PFC samples were homogenized in 1 ml 0.1 M perchloric acid and centrifuged at 14000 rpm for 10 min at 4 °C. The supernatant was removed and 100 µl were injected into a HPLC (High-Performance Liquid Chromatography) column (Gemini C18 110A, 150 × 4.60 mm, 5 u, Bester) connected to a detector (analytical cell: ESA model 5011, 0.34 V). The mobile phase consisted of 62.7 mM Na_2HPO_4, 40.0 mM citric acid, 0.27 mM EDTA, 4.94 mM HSA and 10% MeOH (pH 4.1). Known amounts of 5-HT and 5-HIAA were run in parallel for standardization. Monoamine levels were calculated as ng/g tissue.

2.6. Data analysis

Temperature telemetry data were collected from 60 min before the injection until 120 min after the vehicle or drug injection respectively. For each animal, the average of the values before each challenge was considered as a baseline. After qualitative examination of the data, the end of the response was set at 90 min after the injection of vehicle and 60 min after S-15535 or 8-OHDPAT.

The analysis of the vehicle response was carried out using a Repeated-Measures ANOVA with "Time" (19 levels) as within-subject factor, and "Type" (2 levels: aggressive and non-aggressive) as between-subject factor, in order to examine possible differences that could mask the drug responses.

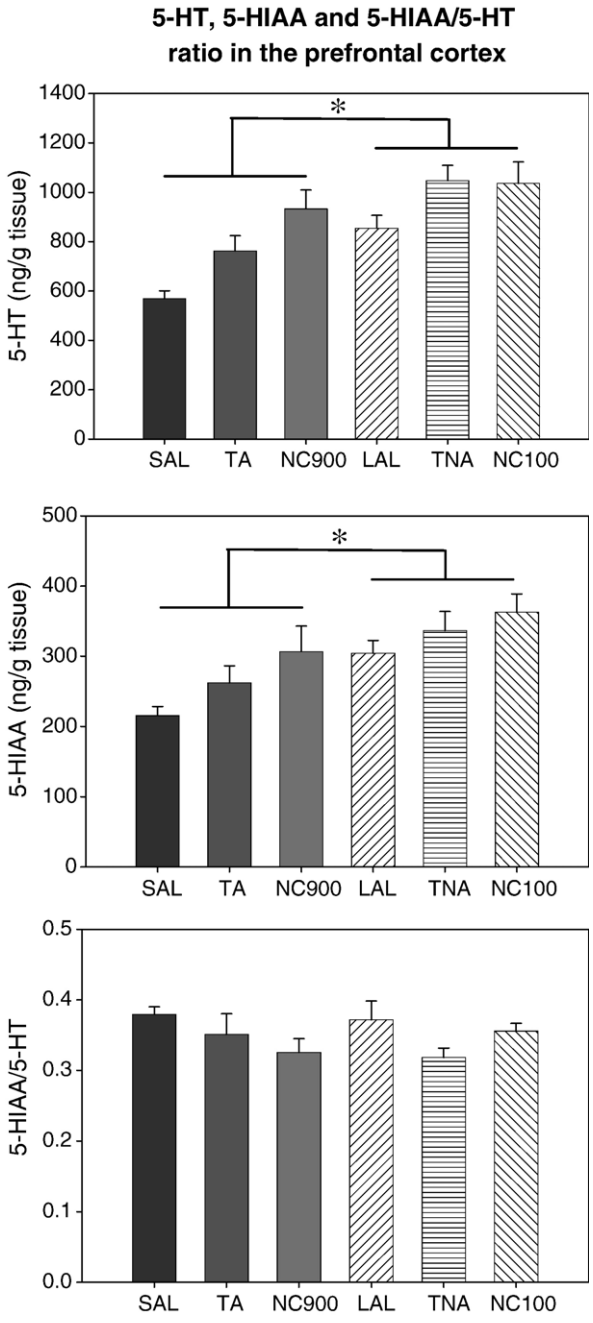

Fig. 2. Amounts of serotonin (5-HT), its metabolite (5-HIAA) and its metabolic ratio (5-HIAA/5-HT) in the prefrontal cortex of mice selected for high (SAL, $n=17$; TA, $n=8$; NC900, $n=8$) and low aggressiveness (LAL, $n=14$; TNA, $n=9$, NC100, $n=9$). *type effect: aggressive vs. non-aggressive different at $p<0.001$.

The time-response data for each drug were then divided by the corresponding time-response data for the vehicle, for each animal. A repeated-measures ANOVA with "Time" (13 levels) as within-subject factor and "Type" (2 levels: aggressive and non-aggressive) as between-subject factor was performed on the corrected data within each selection program. All *post hoc* analyses were performed using a *t*-test for independent samples.

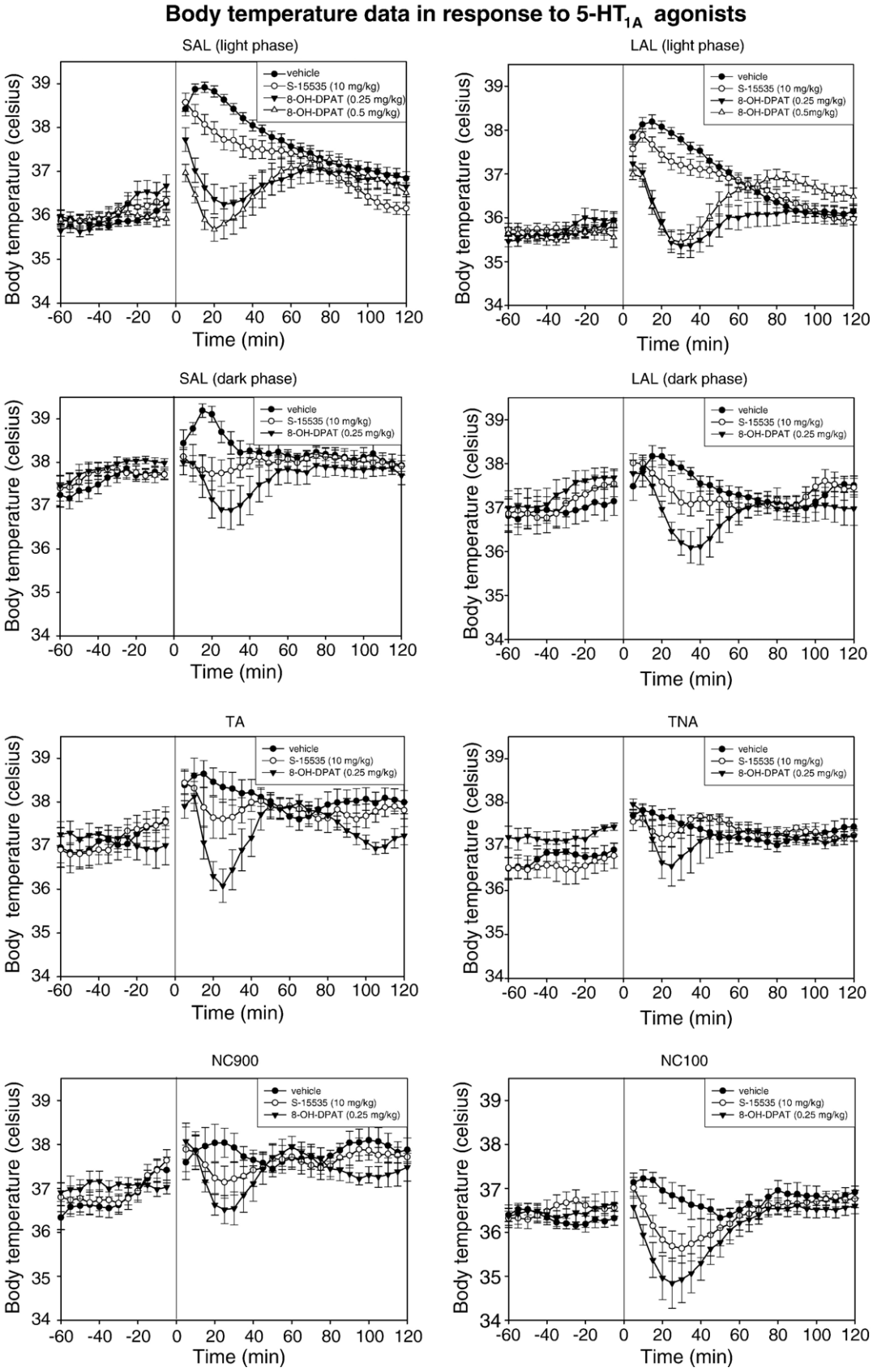

Fig. 3. Body temperature (observed data) of SAL, LAL, TA, TNA, NC900, NC100 mice in response to vehicle (distilled water), S-15535 and 8-OH-DPAT. Data were obtained by means of a telemetry system. Plotted values on the left of the line indicate the measurements before the injection.

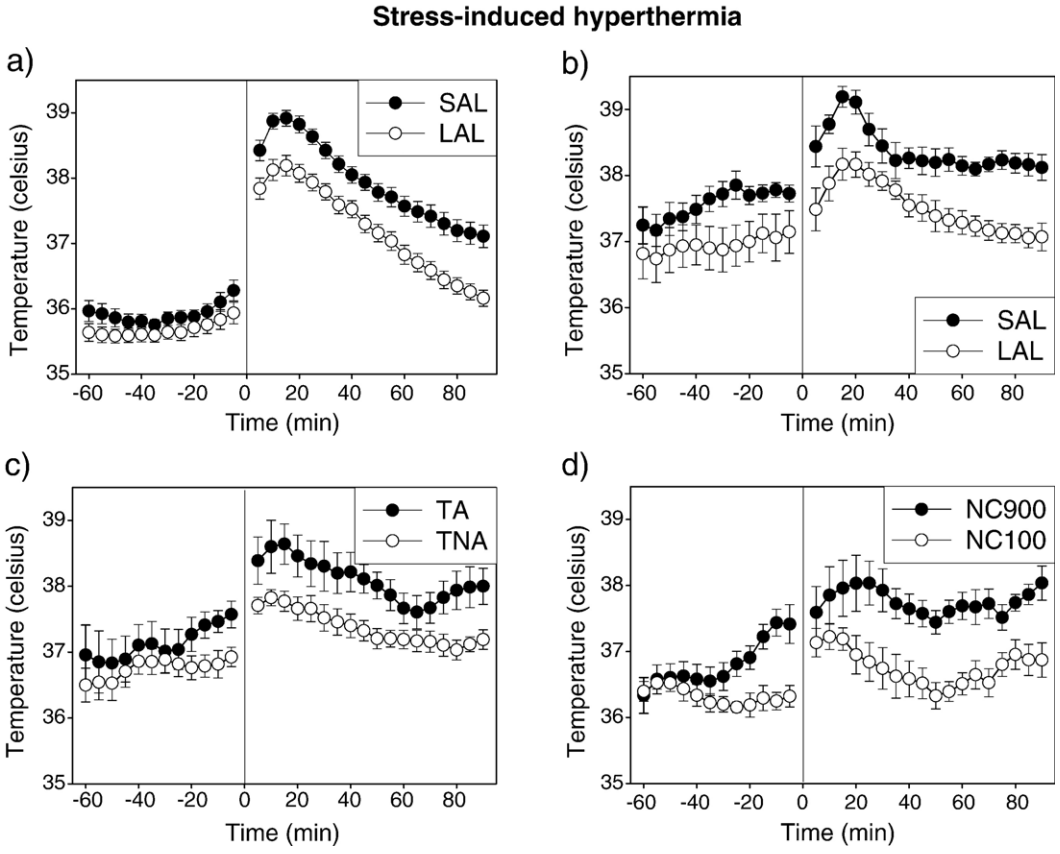

Fig. 4. Body temperature response of mice (closed symbol: aggressive line, open symbol: non-aggressive line) injected with distilled water (5 ml/kg). Graphs represent: a) SAL ($n=30$) and LAL ($n=29$) (light phase); b) SAL ($n=9$) and LAL ($n=8$) (dark phase); c) TA ($n=6$) and TNA ($n=6$); d) NC900 ($n=6$) and NC100 ($n=6$). Data were obtained by means of a telemetry system. Plotted values on the left of the line indicate the measurements before the injection.

The ALT and the behavioral data obtained from the scoring were analyzed using a two-way ANOVA with "type" (2 levels: aggressive, non-aggressive) and "selection" (3 levels: Netherlands, Finland, North Carolina) as between-subject factors.

A similar two-way ANOVA was performed on the HPLC data for 5-HT, 5-HIAA and the 5-HT/5-HIAA ratio.

Post hoc analyses were carried out by means of *t*-tests and Tukey tests for multiple comparisons.

Moreover, for each attacking mouse (from aggressive and non-aggressive lines), AUC ("Area Under the Curve", namely the area comprised between the baseline and the response curve) and Dmax (maximum difference from baseline) values were calculated from the drug/vehicle ratios for S-15535 challenge and used to compute a correlation matrix together with the following variables: 5-HT, 5-HIAA, 5-HIAA/5-HT, ALT and Offense.

All the statistical analyses were carried out using SPSS version 14.0.

3. Results

3.1. Behavioral type confirmation

The ALT test performed on the mice that underwent a surgical procedure confirmed the aggressive and non-aggressive phenotype of almost all the animals. Only one SAL did not attack in 10 min of confrontation. This animal was excluded from the data analysis. The other mice of the aggressive lines had ALT<100 s, with only four mice with ALT between 100 and 150 s. All the attacking mice from the non-aggressive lines (<10% of the LAL, 75% of the TNA) were considered in the data analysis, since their attack latency was >300 s.

3.2. Behavioral data

The average attack latency times for each line are shown in Fig. 1. As expected, the mice selected for aggression attacked much faster than the non-aggressive ones, as revealed by a highly significant "type" effect ($F_{(1,65)}=364.31$, $p<0.001$). The effect was not of the same magnitude in all the strains, as shown by a "selection" effect ($F_{(2,65)}=10.07$ $p<0.001$) and a "selection*type" interaction effect ($F_{(2,65)}=12.39$, $p<0.001$). However, *post hoc* analyses show that in all the three selection breeding programs, the aggressive line was significantly faster than the non-aggressive line (SAL vs. LAL, $t=-38.44$, $p<0.001$; TA vs. TNA, $t=-3.51$, $p<0.01$; NC900 vs. NC100, $t=-56.04$, $p<0.001$).

From all the scored behaviors, eight categories were analyzed and the results are summarized in Fig. 1. A two-way ANOVA revealed significant effects of "type" on *non-social exploration* ($F_{(1,64)}=9.21$, $p<0.01$), *social exploration* ($F_{(1,65)}=76.85$,

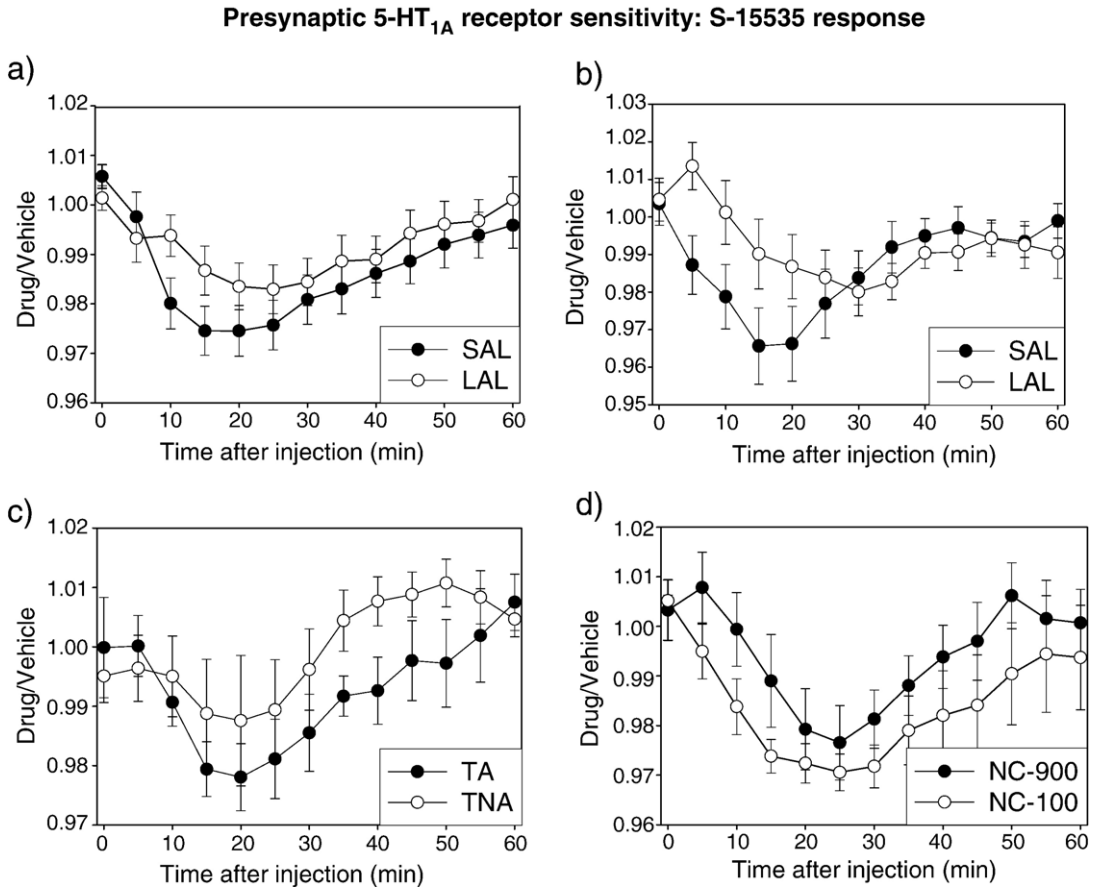

Fig. 5. Body temperature response of mice (closed symbol: aggressive line, open symbol: non-aggressive line) injected with S-15535 (10 mg/kg). Graphs represent: a) SAL ($n=30$) and LAL ($n=29$) (light phase); b) SAL ($n=9$) and LAL ($n=8$) (dark phase); c) TA ($n=6$) and TNA ($n=6$); d) NC900 ($n=6$) and NC100 ($n=6$). Data were obtained by means of a telemetry system and corrected for the vehicle response. $t=0$ is the average value of the 60-min recordings prior to the injection.

$p<0.001$), *offense* ($F_{(1,65)}=279.55$, $p<0.001$), *withdrawal* ($F_{(1,65)}=5.69$, $p<0.05$) and *defense* ($F_{(1,65)}=5.09$, $p<0.05$). The aggressive mice derived from the three different selection breeding programs exhibited similar behavioral characteristics when confronted with an intruder in their home-cage. They spent on average 40–50% of the total test-time in offensive aggression, 20–30% in non-social exploration and much less time in body care, inactivity and withdrawal. The non-aggressive animals spent on average 40% of the confrontation in social exploration, 40% in non-social exploration and little time in body care, inactivity and defense.

3.3. 5-HT and 5-HIAA concentration in PFC

The results of the HPLC on the PFC samples are shown in Fig. 2. Aggressive mice had significantly lower levels of 5-HT and 5-HIAA in the prefrontal cortex, compared to the non-aggressive ones, as shown by the two-way ANOVA ("type" effect: $F_{(1,65)}=18.84$, $p<0.001$). A highly significant "selection" effect ($F_{(2,65)}=10.93$, $p<0.001$) performed on the 5-HT and on the 5-HIAA data. Between the selection programs, the Dutch mice had lower serotonin than the Finnish (Tukey HSD= −200.96, $p<0.001$) and the American mice (Tukey HSD= −288.42, $p<0.001$) and lower 5-HIAA than the American mice (Tukey HSD=−81.71, $p<0.001$). Since no significant "selection*type" interaction effect was found either in the 5-HT or in the 5-HIAA data, we conclude that the differences between aggressive and non-aggressive mice were robust irrespective of the selection breeding program. No significant effects were found in the 5-HIAA/5-HT data.

3.4. Pharmacological challenges

3.4.1. Vehicle response

As shown in Figs. 3 and 4, an injection of distilled water provoked in all the animals a pronounced stress-induced hyperthermia, revealed as a time effect in the ANOVA (SAL vs. LAL in light phase: $F_{(12,180)}=167.3$, $p<0.001$; SAL vs. LAL in dark phase: $F_{(12,180)}=15.08$, $p<0.001$; TA vs. TNA: $F_{(18,180)}=9.3$, $p<0.001$; NC900 vs. NC100: $F_{(18,180)}=3.7$, $p<0.001$). Within the Dutch selection program, in the light phase SAL mice showed an enhanced hyperthermia compared to LAL mice (type effect: $F_{(1,57)}=16.77$, $p<0.001$) with a peak body temperature after 15 min, with SAL temperature higher than LAL ($t=-3.7$, $p<0.001$). The same result is seen in the dark phase (type effect: $F_{(1,15)}=11.65$, $p<0.01$), with SAL responding more than LAL (peak temperature after 15 min: $t=-3.6$, $p<0.01$). TA mice responded more than TNA (type effect: $F_{(1,10)}=7.62$, $p<0.05$).

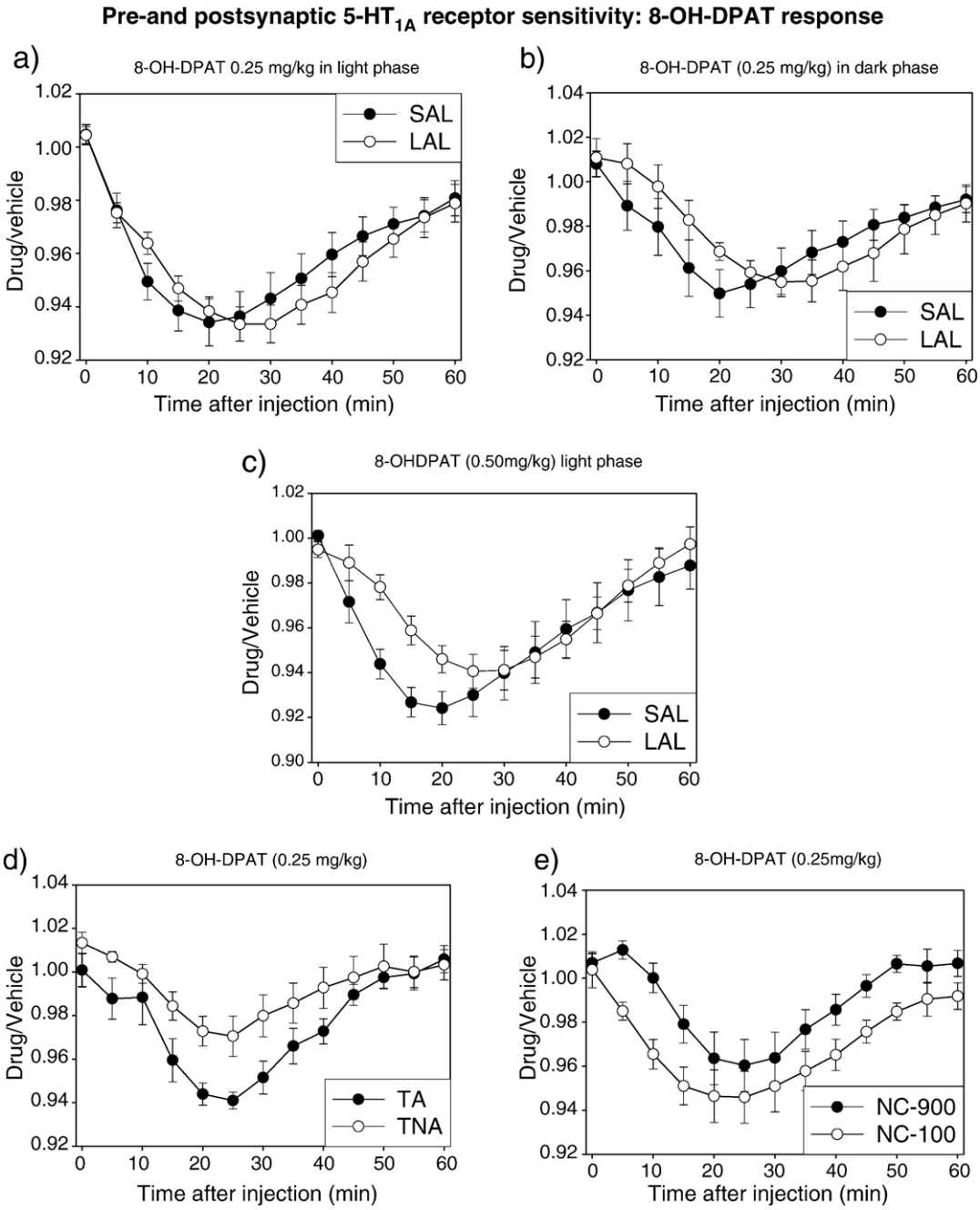

Fig. 6. Body temperature response of mice (closed symbol: aggressive line, open symbol: non-aggressive line) injected with 8-OH-DPAT (0.25–0.50 mg/kg). Graphs represent: (a) SAL ($n=22$) and LAL ($n=20$) (0.25 mg/kg light phase); (b) SAL ($n=11$) and LAL ($n=12$) (0.25 mg/kg dark phase); (c) SAL ($n=9$) and LAL ($n=8$) (0.50 mg/kg, light phase); (d) TA ($n=6$) and TNA ($n=6$) (0.25 mg/kg); e) NC900 ($n=6$) and NC100 ($n=6$) (0.25 mg/kg). Data were obtained by means of a telemetry system and corrected for the vehicle response. $t=0$ is the average value of the 60-min recordings prior to the injection.

North-Carolina mice showed a similar result, with a higher hyperthermia in the aggressive animals (NC900) compared to the non-aggressive ones (NC100) (type effect: $F_{(1,10)}=14.39$, $p<0.01$).

3.4.2. S-15535 response

The response to an injection of S-15535 is illustrated in Figs. 3 (observed values) and 5, in which body temperature was expressed relative to the vehicle. A decrease in the drug/vehicle ratio was observed in all the lines (time effect: SAL vs. LAL, light phase: $F_{(12,264)}=12.62$, $p<0.001$; SAL vs. LAL dark phase: $F_{(12,180)}=4.57$, $p<0.001$; TA vs. TNA = $F_{(12,120)}=3.86$, $p<0.001$; NC900 vs. NC100: $F_{(12,120)}=5.17$, $p<0.001$). A repeated measures ANOVA did not show significant effects on SAL/LAL values in the light phase, but a tendency to a more rapid decrease in SAL than LAL mice was observed, which led to lower values in SAL than in LAL at $t=10$ ($p=0.06$). In the dark phase, a "type*time" interaction effect was found ($F_{(12,180)}=2.8$, $p<0.01$), and SAL had lower values than LAL already after 5 min ($t=2.59$, $p<0.05$). No significant difference was found between TA and TNA or in the NC-lines.

3.4.3. 8-OH-DPAT response

The response to an injection of 8-OH-DPAT is illustrated in Figs. 3 (observed values) and 6, in which body temperature was expressed relative to the vehicle. A marked decrease in drug/vehicle ratio was observed in all the animals (time effect: SAL/LAL, light phase, 0.25 mg/kg: $F_{(12,480)}=51.34$, $p<0.001$; SAL/LAL, light phase, 0.50 mg/kg: $F_{(12.252)}=31.03$, $p<0.001$; SAL/LAL, dark phase, 0.25 mg/kg: $F_{(12,180)}=12.82$, $p<0.001$; TA/TNA, 0.25 mg/kg: $F_{(12,120)}=17.2$, $p<0.001$; NC900/NC100, 0.25 mg/kg: $F_{(12,120)}=14.46$, $p<0.001$). In the light phase, the response to the 8-OH-DPAT (0.25 mg/kg) was faster in SAL than in LAL when the dosage was higher (0.50 mg/kg) ("type*time" effect: $F_{(12,252)}=2.8$, $p=0.001$). The effect was consistent in the dark phase at lower dose (0.25 mg/kg) ("type*time" effect: $F_{(12,180)}=1.9$, $p<0.05$). Regarding the TA/TNA selection, no difference in the speed of the response was found, but an overall higher response in the aggressive line, compared to the non-aggressive one ("type" effect: $F_{(1,10)}=4.8$, $p=0.05$), reaching the maximum difference after 20 min ($t=-3.2$, $p=0.01$). The response of NC900 mice did not differ significantly from the response of NC100 mice.

Table 1
Correlations between 5-HT_{1A} autoreceptor activity, serotonin in PFC and offensive aggressive behavior

		Dmax	AUC	5-HIAA	5-HT	5-HIAA/5-HT	ALT
Dmax	*r*	1					
	p						
AUC	*r*	.857**	1				
	p	.000					
5-HIAA	*r*	.002	−.003	1			
	p	.991	.988				
5-HT	*r*	.314	.306	.819**	1		
	p	.126	.137	.000			
5-HIAA/5-HT	*r*	−.539**	−.526**	.211	−.378	1	
	p	.005	.007	.312	.063		
ALT	*r*	.291	.384	.539**	.536**	−.086	1
	p	.158	.058	.005	.006	.683	
Offense	*r*	−.379	−.411*	−.523**	−.507**	.026	−.680**
	p	.062	.041	.007	.010	.901	.000

r indicates Pearson correlation coefficient.
p indicates p-value.
**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

4. Discussion

Compared to non-aggressive mice, excessively aggressive mice were found to have: (1) enhanced 5-HT_{1A} receptor sensitivity; (2) low amounts of serotonin (5-HT) and its main metabolite, 5-HIAA, in the prefrontal cortex. When considering all the attacking mice, independent of the type of selection program and the strain used for the breeding, serotonin in the prefrontal cortex correlates with, the response to the 5-HT_{1A} autoreceptor agonist and offensive/impulsive aggression, as shown in Table 1. The amount of serotonin in the prefrontal cortex is low in the aggressive mice with higher sensitivity of the 5-HT_{1A} autoreceptor. This suggests that the 5-HT_{1A} autoreceptor inhibition of serotonergic neurons at the level of the raphe nuclei is a major trait characteristic in highly aggressive individuals.

To measure the sensitivity of the 5-HT_{1A} receptor we used pharmacological tools, namely the selective 5-HT_{1A} agonists S-15535 and 8-OH-DPAT. The reference values used in this analysis were the data obtained in response to a vehicle injection. The mice used in our study already showed a pronounced strain difference in the hyperthermic response to the vehicle. Interestingly, within each selection program, all the highly aggressive mice showed higher stress-induced hyperthermia than the low aggressive ones. A rise in body temperature is typically observed in response to stress and is primarily mediated by the autonomic nervous system, particularly with activation of the sympatho-adrenal-medullary branch [38]. In rodents, the release of noradrenaline from sympathetic terminals in the brown adipose tissue and the sympathetic-driven cutaneous vasoconstriction mediate, respectively, enhanced heat generation and reduced heat dissipation, resulting in increased body temperature [39]. Thus, based on the thermal response to stress, it can be concluded that all three aggressive lines of mice show higher sympathetic activation during stress. This corroborates findings in other animals such as rats and humans, in which a higher sympathetic activation during stress response was observed in aggressive individuals [40,41]. Furthermore, this result is consistent with the characteristics of the hostile-impulsive-uncontrolled human type of aggression as defined by Ramirez and Andreu [42], and may be used to experimentally explore this human condition.

As a measure of presynaptic 5-HT_{1A} autoreceptor sensitivity, we used the S-15535 attenuation of stress-induced hyperthermia, a method described previously in rats [19]. S-15535 behaves as a full agonist at the presynaptic 5-HT_{1A} autoreceptor and as a weak partial agonist/antagonist at postsynaptic 5-HT_{1A} receptors [19,30]. In line with its action on somatodendritic 5-HT_{1A} receptors, it exhibits anxiolytic (blockade of Stress-Induced Hyperthermia (SIH)) and anti-aggressive properties without compromising motor and defensive behaviors [19,43]. The overall anti-SIH response to this drug was more pronounced in SAL than in LAL mice, particularly in the dark phase, indicating a higher sensitivity of the 5-HT_{1A} autoreceptors in this line of aggressive mice. No significant differences were found in the other two genetic selection lines.

In order to study the sensitivity of the 5-HT_{1A} postsynaptic receptors, we used 8-OH-DPAT, a selective 5-HT_{1A} full agonist, which causes not only an attenuation of the SIH, but also a dose-dependent pronounced hypothermia below baseline levels [44]. However, in our animals, hypothermia appears only after the correction for the vehicle response. The 8-OH-DPAT-induced hypothermia measured by means of biotelemetry has been described in detail in the rat [45]. However similar telemetry studies in mice are scarce and usually employ rectal temperature measurements that could mask the drug response with the handling/injection stress response, and/or slightly different protocols and/or mice strains [46–51]. Our baseline temperature values, obtained when the animals were left undisturbed in their

home cages, were much lower than the ones reported in those studies in which a biotelemetry system was not used. After the correction for the vehicle, the hypothermia due to the full 5-HT_{1A} agonist 8-OH-DPAT was more pronounced in SAL vs. LAL and in TA vs. TNA indicating a higher sensitivity of this receptor *in vivo* in two out of three aggressive lines. Previous studies in rats and mice similarly showed a higher sensitivity of 5-HT_{1A} receptors in aggressive and impulsive individuals [28,52]. In humans contrasting results have been obtained [53–56].

It has been postulated in the past decades that in rats and humans the 8-OH-DPAT-induced hypothermia is a postsynaptically mediated response in rats and humans [44,57,58], while in mice it is presynaptic [47,59,60]. This is still controversial and the problem remains unsolved. However, in our study we used S-15535 as a powerful tool to elucidate the contribution of the presynaptic 5-HT_{1A} receptor in the regulation of aggressive behavior. The fact that the decrease in temperature due to the S-15535 was not as pronounced as the one caused by 8-OH-DPAT indicates that the 8-OH-DPAT hypothermia is not merely a presynaptic effect, but probably an additive effect of the pre- and postsynaptic 5-HT_{1A} receptors.

In conclusion, 5-HT_{1A} autoreceptors seem to be very important in modulating the behavior of the Dutch mice. In the Finnish selection program, the postsynaptic 5-HT_{1A} receptor seems to be more important than the presynaptic one in modulating aggressive behavior. We could not replicate these results in the NC900/NC100 mice, but some considerations about these animals should be made. First, NC100 mice were significantly heavier than NC900 (mean body weight (g) ±SEM: SAL=21.7±0.5; LAL=21.6±0.6; TA=31.6±0.6; TNA=31.9±0.4; NC900=36.2±0.9; NC100=49.3±1.9; Tukey's test NC900 vs. NC100 different at $p<0.001$), which seems to be due to more pronounced fat deposits. If this is a sign of some kind of metabolic dysfunction, it could generate a discrepancy in the pharmacodynamics–pharmacokinetics of the drugs injected and it would lead us to erroneous interpretation of the data. Moreover, since we injected the drugs according to the body weight of the animals, the NC100 mice received a higher absolute dose compared to the NC900, which could explain the more pronounced response to both challenges. This topic requires further examination, for instance various metabolic parameters should be checked in these animals, in order to unravel possible confounding variables that might have affected our data.

From the behavioral analysis, we can conclude that all the mice from aggressive lines showed high levels of aggressive behavior (in terms of offensive aggression time) and impulsivity (expressed by attack latency time). The aggressive behavior in the Turku and North Carolina aggressive mice, which were selected with testing and breeding procedures different from the Dutch ones and from different founder strains [20–22], is therefore very stable through generations. It does not require isolation and is comparable to that of the Dutch aggressive mice in the same testing conditions.

Surprisingly, the involvement of serotonin differs slightly between strains, not only in terms of the involvement of the pre- and postsynaptic 5-HT_{1A} receptor, but also regarding the amount of serotonin and its metabolite. From the biochemical assay data, the Dutch mice had lower serotonin concentration in the PFC than the Finnish mice and the North-Carolina mice, and lower metabolite concentration than the North-Carolina mice. As a general conclusion, these results indicate that there are strain differences in the relationship between aggressive behavior and 5-HT_{1A}-receptor system. Aggressive behavior is a functionally important and adaptive form of social behavior in many mammalian species, including humans. While human studies generally concentrate on violence as the pathological expression of aggressive behavior, animal studies are generally based on non-pathological, functional forms of aggression. This discrepancy between normal and pathological aggression may explain the strain differences in our study and the contradictory results on serotonergic functioning in aggression obtained in animal and human studies.

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